IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:		**Customer No.:**	**23448**
Applicant:	**DeVico, et al.**	**Docket No.:**	**4115-144**
Application No.:	**09/684,026**	**Examiner:**	**U. Winkler**
Filed:	**October 6, 2000**	**Art Unit:**	**1648**
Title:	**VIRUS COAT PROTEIN/RECEPTOR CHIMERAS AND METHODS OF USE**	**Confirmation No.:**	**3193**

DECLARATION UNDER 37 CFR §1.131 IN U.S. PATENT APPLICATION NO. 09/684,026

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

ANTHONY L. DEVICO, TIMOTHY R. FOUTS, ROBERT G. TUSKAN, severally and jointly hereby declare:

1. THAT we are co-inventors of the subject matter disclosed and claimed in United States Patent Application No. 09/684,026 filed October 6, 2000 in the United States Patent and Trademark Office in the names of Anthony L. DeVico, Timothy R. Fouts and Robert G. Tuskan and entitled, "VIRUS COAT PROTEIN/RECEPTOR CHIMERAS AND METHODS OF USE," hereafter referred to as the "Application," wherein the Application claims priority to U.S. Provisional Application No. 60/158,321 filed on October 8, 1999, hereafter referred to as the "Provisional Application."

2. THAT the Application discloses and claims chimeric polypeptides comprising a virus coat polypeptide sequence and viral receptor polypeptide sequence that are linked by an amino acid spacer, wherein the spacer is positioned between the virus coat polypeptide and the viral receptor polypeptide and linked thereto to form a single chain polypeptide. The amino acid sequence is of a sufficient length to allow folding of the single chain polypeptide to form an

intramolecular interacting complex between the virus coat polypeptide and the viral receptor polypeptide.

3. THAT we are aware that the Application has been examined by the United States Patent and Trademark Office, and that we are aware that the currently pending claims of the Application have been rejected on various grounds including the disclosure of Chackerian, et al. (Proceedings of the National Academy of Sciences, March 2, 1999 considered the mailing date of the publication).

4. THAT we have been informed by our legal representatives that the rejections of the claims of the Application can be overcome by presenting evidence to the United States Patent and Trademark Office of our possession of the presently claimed invention prior to the effective date of the reference identified in Paragraph 3, that said effective date has been identified to us by such legal representatives as March 3, 1999 (such date hereafter being referred to as "Effective Date" which is the earliest date the publication could be received by the public in light of the fact that this publication was not accessible to the public through other means than mailing and the publication was not published online).

5. THAT attached in Exhibit 1 (Appendix A) hereof is a true and exact copy of pages 1-4 of an Invention Disclosure Document, on which all dates have been blacked out, but which dates are prior to the Effective Date; that page 1 identifies co-inventors Anthony L. DeVico, Timothy R. Fouts and Robert G. Tuskan, that the title of the document is "Single chain HIV gp120-CD4 chimeric complexes as anti-HIV immunogens and therapeutics," that page 2, second paragraph, second last sentence discusses the "the CD4 and gp120 are linked by 20 amino acid spacer between the C-terminus of gp120 and(derived from the HIV-1 isolate BaL) and the N-terminus of the first two Ig domains of human CD4"; that page 2, under Section 3 discusses the production of the single chain chimeric complex and that in fact the "single chain protein folded into a conformationally altered state indicative of gp120-CD4 complex formation.

6 THAT we offer Exhibit 1 with this Declaration as evidence of the completion and possession of the single chain chimeric polypeptides disclosed and claimed in the Application and Provisional Application prior to the Effective Date identified in Paragraph 4 of this Declaration.

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like, so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Anthony L. DeVico — Date 7/28/04

Timothy R. Fouts — Date 8/24/04

Robert G. Tuskan, — Date _____

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like, so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

_____ Date_____

Anthony L. DeVico

_____ Date _____

Timothy R. Fouts

Date 07-29-2004

Robert G. Tuskan,

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INVENTION DISCLOSURE FORM
Invention Disclosure Number: [redacted]
INVENTION
TITLE: "Single chain HIV gp120-CD4 chimeric complexes as anti-HIV immunogens and therapeutics."

1. INVENTOR INFORMATION

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INVENTION DISCLOSURE

2. STATEMENT OF INVENTION AND SUGGESTED SCOPE

Give a complete description of the inventions. If necessary, use additional pages, drawings, diagrams,etc. Description may be by reference to a separate document (copy of a report, preprint, grant application and the like) attached hereto. If so, identify the document positively.

Gp120, a subunit of the envelope protein of HIV-1, binds to CD4 and undergoes a conformational change to form a "transition state" that permits the complex to interact with a chemokine receptor, such as CCR5. This interaction permits the infection of HIV-1 into target CD4+ cells. Antibodies that interfere with the interaction of HIV-1 with the chemokine receptor can prevent infection. Immunogens capable of generating such antibodies may make a effective preventive vaccine against HIV-1. However, the construction of such immunogens is currently problematic. The construction of soluble protein complexes involves the combination of two soluble components, with highly variable results. In the context of DNA vaccines, the coordinated expression of equimolar concentrations of each component is problemati, especially since the gp120 gene is under the control of HIV regulatory proteins. To solve these problems, we constructed a gene that produces the two key components of the interaction, CD4 and gp120, as a single amino acid chain. The gp120 gene is altered to encode the correct amino acid sequence with human codons, and is thus expressed independent of HIV regulatory proteins. The CD4 and gp120 are linked by 20 amino acid spacer between the C-terminus of gp120 (derived from the HIV-1 isolate BaL) and the N-terminus of the first two Ig domains of human CD4. This spacer sequence is long enough to permit the interaction of CD4 and gp120. The entire chain is tagged 3' to the sCD4 sequence with a short peptide sequence derived from the c-myc oncogene in order to conveniently detect and characterize the molecule.

There are three potential uses for this invention. 1) The epitopes that become exposed upon CD4 binding, some of which interact directly with the chemokine receptor, are stabilized in this construct without the use of chemical crosslinkers. Optimal exposure of these epitopes, without crosslinkers, enhances the potential of inducing neutralizing antibodies against HIV-1 in experimental animals and humans. This single chain CD4/gp120 molecule is therefore a potential vaccine against HIV-1 either as a subunit vaccine or as a component of vaccine delivery system. 2) The molecules are HIV corecpetor anatgonists and might be used therapeutically to block HIV spread. 3) the molecules could be used to screen for other HIV coreceptor antagonists that act to competitively inhibit HIV envelope-CD interactions with coreceptors.

3. RESULTS DEMONSTRATING THE CONCEPT IS VALID

Cite specific results to date. Indicate whether you have completed preliminary research studies, laboratory model or prototype testing. Attach additional pages if necessary or reference specific experimental results in a separate attached document.

The single chain protein was expressed by transient transfection of human cell lines and the resulting protein characterized. Immunoblotting techniques demonstrated that the protein expressed was approximately 145 kDa, the expected size for the a single chain molecule comprised of sCD4 and gp120. This 145 kDa protein was recognized by antibodies specific for either sCD4, gp120 or myc. Two additional techniques were then utilized to assess whether the single chain protein folded into a conformationally altered state indicative of gp120-CD4 complex formation. First, enzyme-linked immunoassays (ELISA) using antibodies specific for epitopes on gp120 that become exposed only after

binding to sCD4, demonstrated that these conformationally-dependent epitopes are highly exposed in the single chain protein. Second, the single chain molecule binds to a murine B-cell lin eexpressing only human CCR5 but not CD4, as expected for a gp120-CD4 comlex as determined by FACS (flourescent-activated cell sorting). Taken together, this data demonstrates that the CD4 and gp120 components of the single chain molecule are interacting and folding into a stable CD4-gp120 complex that retains the appropriate conformationally altered structure .

4. VARIATIONS AND ALTERNATIVE FORMS OF THE INVENTION

State all of the alternative forms envisioned to be within the full scope of the invention. List all potential forms of the invention, whether currently proven or not.

This scope of this invention encompasses any modification of the aforementioned gene sequence. Such alterations can include, but are not limited to,

(1). deleting conserved or variable regions of the gp120 component,
(2). deleting or adding amino acids within the gp120 component that would alter the glycosylation pattern of the molecule, which may be critical in enhancing immunogenicity
(3). deleting or extending of the amino acid spacer region,
(4). expanding the size of the sCD4 component to include the two other Ig ectodomains of human CD4,
(5). genetically switching the gp120 component to a gp120 or gp140 derived from another HIV-1, HIV-2, or SIV isolate,
(6). genetically switching the sCD4 component to a sCD4 derived from rhesus macaques or some other primate species,
(7). deleting, extending, switching or altering the peptide tag sequence derived from the c-myc oncogene.
(8). attaching the single chain moiety either genetically or chemically to an Ig molecule or some carrier protein such as ovalbumin.

5. NOVEL FEATURES

a. Specify the novel features of your invention. How does the invention differ from present technology?

b. What is the deficiency in the present technology which your invention improves upon?

(1). This gene drives the expression of sCD4 and gp120 as a single amino acid chain. Current technology requires that the individual sCD4 and gp120 be purified singly and mixed at optimal concentrations to facilitate complex formation. The resulting complexes must then be chemically crosslinked to retain their conformationally altered state. By expressing the protein as a single amino acid chain, the numerous steps involved in purification, complexing, and crosslinking of the individual proteins are avoided. As a single chain, the sCD4 and gp120 components are expressed de novo in concentrations optimal for complex formation. These preformed complexes can then be purified and used.
(2). The CD4 and gp120 are genetically linked by 20 amino acid spacer between the C-terminus of gp120 (derived from the HIV-1 isolate BaL) and the N-terminus of the first two Ig domains of human CD4. This spacer sequence is long enough to permit the interaction of CD4 and gp120. This facilitates the expression of gp120-CD4 complexes in DNA vaccines.
(3). The entire chain is tagged with a short peptide sequence derived from the c-myc oncogene. This peptide tag is encoded in a short DNA sequence 3' to the sCD4 gene sequence. This peptide tag can be used to purify the single chain complexes.
(4). Epitopes that become exposed upon CD4 binding, some of which interact directly with the chemokine receptor, are stabilized in this construct without the use of chemical crosslinkers.
(5). The gp120 gene has been genetically altered to optimize expression in mammalian cell lines. This has been achieved by changing the codons encoded in the native gene sequence to those used most frequently by mammalian cells.
(6). The spacer region includes useful restriction enzyme sites to optimize future genetic alterations of the sequence
(7) The invention is the first recombinant molecule ever developed that represents a gp120 "transition state" that is competent to bind a coreceptor.

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6. APPLICATIONS OF THE TECHNOLOGY

List all products you envision resulting from this invention and whether these products can be developed in the near term (less than two years) or long term.

(1). Purified soluble protein that can be used as

(a). a preventative vaccine against HIV-1. This protein can be used either alone or as a soluble protein boost in combination with some other vaccine strategy.

(b). a means to define candidate anti-HIV therapeutics that block the single chain from interacting with its intended chemokine receptor. Such reagents could include but are not limited to organic compounds, antibodies, peptides, etc.

(c) an antiviral agent to prevent coreceptor interactions with HIV.

(2). The gene can be included in a vaccine delivery vehicle that expresses the protein upon introduction into the animal host. Such vehicles include but are not restricted to plasmids used as DNA vaccines, attenuated viruses such as vaccinia ankara, lipid formulations that deliver DNA vaccines, or bacteria that either express the protein directly or transmit DNA that can drive the expression of the protein in mammalian cells. Such delivery vehicles can be used either as preventative vaccines alone or in combination with some other vaccine strategy.

7. SUPPORTING INFORMATION

a. Are there publications, reports, preprints, etc. pertaining to the invention? Please list publication dates. Include manuscripts for publication (submitted or not), new releases feature articles and items from internal publications.

None at this time.

b. What was the date the invention was first conceived? Is this date documented? Where? Give reference numbers and physical location of lab records, but do not enclose?

[redacted]